2206-Pos Board B225
Solid-State NMR and Fluorescence Spectroscopy of Antimicrobial Methylated-Tryptophan Lactoferricin Peptides with Gln, Gly or Pro as the Central Residue
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In recent years pathogenic bacteria have become increasingly resistant to common antibiotics, leading to a demand for new, more potent antibacterial drugs. The 25 amino acid peptide lactoferricin (LfB25) exhibits broad spectrum antimicrobial properties and is of interest for drug development. A cationic amidated hexapeptide, (LfB6: **RRW**Q**WR**-NH_2), containing three arginines and two tryptophans has been identified as the ''antimicrobial active site.'' Our lab has shown that the antimicrobial activity of LfB6 can be enhanced by methylation of the tryptophan indole nitrogen (MeTrp). After selective deuteration of the MeTrp residues, membrane interactions can be examined by solid-state ^{2}H NMR spectroscopy in oriented bilayers composed of mammalian- and bacterial-like membranes. A symmetric heptapeptide with four arginines and two methyl-tryptophans (RR**MeW**Q**MeW**RR-NH_2; LfB7 MeTrp 3,5) was designed and found to increase antimicrobial activity relative to LfB6. ^{2}H NMR spectra reveal that the MeTrp residues are aligned at the membrane surface, while ^{31}P NMR spectra indicate very little perturbation to the lipid head groups. Partitioning assays in multilamellar vesicles demonstrate an increase in membrane binding correlated with percent anionic lipid, and Trp emission fluorescence spectroscopy confirms that the MeTrps are more water exposed in neutral compared to anionic lipid membranes. To investigate the importance of backbone flexibility for activity and membrane interaction, the central glutamine of the heptapeptide has been changed to either Gly or Pro. Initial results indicate that the Pro-constrained peptide, RRMeW**P**MeWRR**,** is less antimicrobial and has weaker membrane alignment and binding than when Gln is the central residue. We will present results for the more flexible peptide with Gly as the central residue, RRMeW**G**MeWRR.

2207-Pos Board B226
Identifying Steroid Hormone Binding Sites on Human Serum Albumin by 2D NMR
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Steroid hormones, such as testosterone and estradiol, are often prescribed to the aging population as an attempt to combat symptoms associated with lower hormone levels (*i.e.* poor sex drive, weight gain, and fatigue). In human serum, these hormones bind reversibly to the sex hormone binding globulin (SHBG) and to a lesser extent to albumin. The specific sites on albumin and whether hormone binding alters fatty acid (FA) binding have not been established, properties which both could affect delivery to cells and the choice of the appropriate dosage of the hormone to give the desired therapeutic effect. To study binding to human serum albumin (HSA), we examined displacement of ^{13}C-methyl-enriched oleic acid (OA) complexed with HSA in 2D NMR spectra that resolve 9 binding sites and quantify their relative affinities for OA. NMR studies were conducted within physiologically relevant concentrations of HSA, at a molar ratio of 4:1 OA:HSA, and with non-physiological levels of hormone. Testosterone and estradiol displaced OA in a low affinity site for FA, which we previously identified as Sudlow's Drug Site 2. Testosterone also partially displaced OA at FA Site 6, a low affinity FA binding site and a secondary site for certain drugs, as identified by NMR and x-ray. Neither hormone displaced FA in the medium affinity site corresponding to Sudlow's Drug Site 1. Our findings show that these steroids can bind to albumin in sites that are not likely to interfere with FA binding under physiological conditions with molar ratios that are less than 4:1 OA:HSA.

2208-Pos Board B227
Comparision of Membrane Interactions of Acylated and Non-Acylated Lactoferricins by Solid-State NMR Spectroscopy and Molecular Dynamics Simulations
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LfB6 (RRWQWR-NH2) is a tryptophan- and arginine-rich cationic antimicrobial peptide with broad spectrum activity derived from bovine lactoferrin. Membrane binding occurs via electrostatic interactions between arginines and negative charges on the bacterial cell membrane and intercalation of the tryptophans at the membrane interface. N-terminal acylation ($CH_3(CH_2)_4CO$-RRWQWR-NH_2; C6-LfB6) can enhance the antimicrobial activity (Greathouse et al. (2008) J. Pept. Sci. 14:1103). Solid-state ^{2}H and ^{31}P NMR spectroscopy combined with all-atom and coarse-grained molecular dynamics (CG-MD) simulations have confirmed subtle differences between 1 mol% LfB6 and C6-LfB in bilayers composed of 3:1 POPE:POPG (anionic, bacterial-like) and POPC (zwitterionic, mammalian-like). MD simulations reveal that the arginines of C6-LfB6 make first contact with POPE:POPG; whereas the C6 tails are first to contact POPC. LfB6 shows no sequence preference. Additionally, C6-LfB6 inserts more deeply than LfB6 into both membranes. Tryptophan emission fluorescence spectra suggest the tryptophans in LfB6 and C6-LfB6 are more water exposed in neutral compared to anionic membranes, while CG-MD simulations reveal that LfB6 comes off the POPC membrane, exposing the tryptophans to water. Acylation, therefore, increases the ''stickiness'' of the peptide for lipid bilayers. Although both peptides at 1 mol% show significant membrane effects during short range simulations, C6-LfB6 has less influence on lipid order. We now compare experimental and molecular dynamics results for LfB6 and C6- LfB6 at 4 mol%. Solid-state ^{2}H NMR spectra indicate that C6-LfB6 has a greater effect on the lipid acyl chain order at 4 mol% compared to 1 mol%; whereas the effects of LfB6 are similar at both concentrations. Molecular dynamics simulations will be presented for comparison.

2209-Pos Board B228
Differential Interaction of Bovine Alpha-Lactalbumin with Membranes: Interplay of Negatively Charged Lipids and Cholesterol
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Many soluble proteins are known to interact with membranes and the mechanism of such interactions in cellular processes is beginning to be understood. Bovine alpha-lactalbumin (BLA) is a soluble protein and yet it interacts with membranes. We recently reported the specific binding of apo-BLA with negatively charged membranes using a variety of fluorescence approaches [1]. A novel finding is that BLA exhibits an enhanced binding to negatively charged membranes in the presence of cholesterol and it possesses cholesterol recognition/interaction amino acid consensus (CRAC), a motif recognized for preferential association with cholesterol in many proteins. We monitored the specificity of sterol interaction by replacing cholesterol with 7-dehydrocholesterol (an immediate biosynthetic precursor of cholesterol), and observed that BLA-sterol interaction is specific to membrane cholesterol. Significant Fluorescence Resonance Energy Transfer (FRET) was observed between the tryptophan residues of BLA and dehydroergosterol (a naturally occurring fluorescent analog of cholesterol) in presence of negatively charged membranes, indicating close proximity between them. Dipole potential measurements upon BLA binding to membranes and docking studies of cholesterol with BLA provide useful insight into the lipid selectivity of BLA binding to membranes. Depth analysis by the parallax approach provides evidence for interfacial localization of tryptophans of BLA when bound to membranes. Taken together, our results assume significance in the light of tumoricidal and antimicrobial functions of α-lactalbumin [2,3].
References:
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2210-Pos Board B229
Exploring Rhodopsin-Bilayer Interactions via Coarse-Grained Molecular Dynamics Simulation
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Proteins are dynamic in structure, with molecular motions dictated primarily by local physical forces. Integral membrane proteins differ in the sense that the environment plays a major role in protein flexibility and, in turn, function. Rhodopsin, a G protein-coupled receptor, is a membrane protein whose function is dependent on major environmental factors, including lipid composition, cholesterol concentration, and the ionic strength of the surrounding solvent. In this work, we further explored these effects by utilizing coarse-grained molecular dynamics to simulate large, native-like membranes for long timescales. We discovered clear preferences at the surface of the protein for polyunsaturated lipid tails, an effect that has been explored before with simulation, though not at the timescales present in this work. We also noted preferential binding regions for cholesterol, possibly suggesting specific binding sites.

2211-Pos Board B230
Computational Studies of Talin-Mediated Integrin Activation
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Integrins are heterodimeric (αβ) cell surface receptors that are involved in many essential cellular processes, such as cell migration, as well as